Supplemental Table 1: Main Inclusion and Exclusion Criteria

Inclusion criteria • Age 15 years or older • Clinically manifested vision loss in at least one eye, due to *MT-ND4* LHON • Documented results of genotyping showing the presence of the m.11778G>A mutation in the *ND4* gene and absence of other primary LHON-associated mutations (m.3460G>A or m.14484T>C) • Duration of vision loss ≤1 year in each affected eye • At least Hand Motion visual acuity in each eye • Negative test for human immunodeficiency virus infection • Negative pregnancy test for women of childbearing potential • Female subjects (if of childbearing potential) had to agree to use effective methods of birth control and male subjects had to agree to use condoms, for up to 6 months after treatment administration • Discontinuation of idebenone treatment at least 7 days before inclusion. Concomitant treatment with idebenone was not authorized during the study
Exclusion criteria • Any known allergy or hypersensitivity to lenadogene nolparvovec • Contraindication to IVT in any eye • Previous treatment with an investigational medicinal drug (except idebenone if discontinued at least 7 days before inclusion) • Ocular surgery of clinical relevance (per investigator assessment) within 90 days preceding the screening • IVT performed in any eye within 30 days prior screening • Disorders of the eye or adnexa other than LHON which may interfere with ophthalmologic assessments (including SD-OCT) during the study • Mutations other than m.11778G>A in the *ND4* gene known to cause pathology of the optic nerve, retina or afferent visual system • Systemic or ophthalmologic disorders other than LHON known to be associated with visual dysfunction or for which associated treatment(s) may cause visual dysfunction • History of recurrent uveitis or active ocular inflammation • Presence of alcoholism or drug abuse (excluding nicotine)

IVT, intravitreal; LHON, Leber Hereditary Optic Neuropathy; SD-OCT, Spectral-Domain Optical Coherence Tomography

Supplemental Table 2: Baseline Characteristics of REFLECT Patients

	N=98 subjects (196 eyes)	
	Mean (SD) or n (%)	**Range**
DEMOGRAPHIC CHARACTERISTICS		
Gender, Male, n	78 (79.6%)	
Age at onset of the disease (years)	31.5 (13.8)	14; 73
Age group at onset of the disease, n		
<15 years	4 (4.1%)	
15-18 years	9 (9.2%)	
18-60 years	80 (81.6%)	
≥60 years	5 (5.1%)	
Age at screening (years)	32.1 (13.8)	15; 74
Age group at screening, n		
15 – 18 years	10 (10.2%)	
18 – 60 years	83 (84.7%)	
≥60 years	5 (5.1%)	
Study region (location of study sites), n		
Asia	15 (15.3%)	
Europe	27 (27.6%)	
United States	56 (57.1%)	
Current tobacco smoking, n	21 (21.4%)	
Current alcohol consumption, n	48 (49.0%)	
Blood Pressure		
Systolic Blood pressure (mm Hg)	127.7 (14.61)	92, 166
Diastolic Blood Pressure (mm Hg)	80.1 (10.83)	54, 110
Medical history, n[1]		
Anxiety	15 (15.3%)	
Hypertension	14 (14.3%)	
Cataract	13 (13.3%)	
DISEASE CHARACTERISTICS		
Duration of vision loss at baseline (months)	8.30 (3.2)	1.7; 11.9
Inter-eye delay of vision loss (months)	1.95 (1.98)	0; 8.7
Simultaneous vision loss of both eyes	24 (24.49%)	
Bilateral vision loss at baseline[2], n	97 (99.0%)	
Prior idebenone treatment[3], n	14 (14.3%)	
Best Corrected Visual acuity (BCVA)		

BCVA of all eyes	1.55 (0.47)	0; 2.30
BCVA of first-affected eye (LogMAR)	1.64 (0.45)	0.60; 2.30
BCVA of second/not-yet-affected eye (LogMAR)	1.47 (0.48)	0.00; 2.30
Better seeing-eye, n		
First affected eye	27 (27.6%)	
Second/not-yet-affected eye	71 (72.4%)	
Eyes off-chart at baseline (BCVA >1.6 LogMAR)		
First-affected eye	40 (40.8%)	
Second/not-yet-affected eye	28 (28.6%)	
Both eyes off-chart	25 (25.5%)	
Intraocular Pressure (IOP)	14.92 (2.9)	6; 22
Contrast sensitivity (LogCS)		
LogCS of all eyes	0.37 (0.46)	0; 1.35
LogCS of first affected eye	0.31	0; 1.35
LogCS of second/not-yet-affected affected eye	0.44	0; 1.35
Humphrey Visual Field		
HVF mean deviation (MD) (dB)		
HVF MD of all eyes (dB)	-22.31 (10.3)	-34.6; -0.3
HVF MD of first affected eye (dB)	-23.25	-34.6; -0.3
HVF MD of second affected eye (dB)	-21.37	-34.5; -1.6
HVF pattern standard deviation (PSD) (dB)		
HVF PSD of all eyes (dB)	7.02 (3.5)	1.4; 14.0
HVF PSD of first affected eye (dB)	6.91	1.5; 14.0
HVF PSD of second affected eye (dB)	7.13	1.4; 13.6
HVF foveal threshold (FT) (dB)[4]		
HVF FT of all eyes (dB)	6.05 (10.1)	0; 43.0
HVF FT of first affected eye (dB)	5.86	0; 36.0
HVF FT of second affected eye (dB)	6.24	0; 43.0
STRUCTURAL PARAMETERS (SD-OCT)[5]		
GCL macular volume (mm^3)		
GCL macular volume of all eyes (mm^3)	0.60 (0.1)	0.35; 1.00
GCL macular volume of first affected eye (mm^3)	0.58	0.38; 0.93
GCL macular volume of second affected eye (mm^3)	0.63	0.35; 1.00
Papillo-Macular Bundle (PMB) RNFL thickness (µm)		
PMB RNFL thickness of all eyes (µm)[6]	29.5 (13.4)	10.0; 126.0
PMB RNFL thickness of first affected eyes (µm)	28.17	10.0; 126.0

PMB RNFL thickness of second affected eyes (µm)	30.77	10.0; 101.0
Temporal Quadrant RNFL thickness (µm)[6]		
Temporal Quadrant RNFL thickness of all eyes (µm)	38.38 (23.0)	19.0; 183.0
Temporal Quadrant RNFL thickness of first affected eyes (µm)	34.99	19.0; 183.0
Temporal Quadrant RNFL thickness of second affected eyes (µm)	41.69	19.0; 162.0
Average RNFL thickness (µm)[6]		
Average RNFL thickness of all eyes (µm)	80.85 (28.97)	37; 200
Average RNFL thickness of first affected eyes (µm)	76.09	38; 197
Average RNFL thickness of second affected eyes (µm)	75.50	37; 200
ETDRS macular volume (mm^3)		
ETDRS macular volume of all eyes (mm^3)	7.91 (0.5)	6.8; 9.5
ETDRS macular volume of first affected eyes (mm^3)	7.91	6.8; 9.5
ETDRS macular volume of second affected eyes (mm^3)	8.03	6.2; 9.5

Data presented are mean (SD) and range or counts (%) where appropriate.

[1] Medical history occurring in more than 10% of subjects

[2] One subject was unilaterally affected (only one eye with drop of vision) at baseline

[3] Use of idebenone within 30 days prior to intravitreal injection of the study treatment and discontinued prior to study treatment. Idebenone had to be discontinued at least 7 days prior to inclusion and was prohibited during the study.

[4] 186 eyes (missing data for 5 eyes)

[5] Average of screening and baseline values

[6] 194 eyes (missing data for 2 eyes)

Better-seeing eye: The better-seeing and worse-seeing eye of each subject were determined based on the baseline vision testing performed at inclusion. A pre-defined algorithm for determining the better- and worse-seeing eyes was utilized as follows: 1) LogMAR BCVA (Criterion 1): The eye with the better (i.e., lower) LogMAR BCVA was the better-seeing eye. If both eyes had an equal LogMAR acuity, the second criterion was utilized; 2) SD-OCT parameters (Criterion 2; used if there was no inter-eye difference based on Criterion 1); 3) Log of Contrast Sensitivity (LogCS) (Criterion 3; used if there was no inter-eye difference in criteria 1 or 2). The eye with the better LogCS score was the better-seeing eye; 4) If the better-seeing eye could not be determined based on criteria 1 through 3, the selection of the better-seeing eye was based on the subject's opinion.

BCVA, Best Corrected Visual Acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; GCL, Ganglion Cell Layer; HVF, Humphrey Visual Field; LogCS, Logarithm of Contrast Sensitivity; LogMAR, Logarithm of the Minimal Angle of Resolution; n, number of subjects; RNFL, Retinal Nerve Fiber Layer; SD, Standard Deviation; SD-OCT, Spectral-Domain Optical Coherence Tomography.

Supplemental Table 3. Individual data of a female patient aged between 35 and 40 years with no vision loss in one eye at baseline. The duration of vision loss in the affected eye (left) was 70 days.

		Period	Affected eye	Unaffected eye
BCVA (LogMAR) (Snellen)		Screening	0.7 20/100	0 20/20
		Baseline	0.7 20/100	0 20/20
CS (LogCS)		Screening	1.20	1.65
		Baseline	1.05	1.35
HVF (dB)	**MD**	Screening	-2.64	-4.05
		Baseline	-3.28	-2.82
	PSD	Screening	1.86	2.44
		Baseline	4.17	1.59
SD-OCT	**GCL Macular Volume (mm^3)**	Screening	0.84	0.96
		Baseline	0.86	0.96
	PMB RNFL Thickness (µm)	Screening	53	63
		Baseline	88	68
	RNFL Quadrant Temporal (µm)	Screening	82	87
		Baseline	111	90
	RNFL Average Thickness (µm)	Screening	120	112
		Baseline	129	114
	ETDRS Total Macular Volume (mm^3)	Screening	9.49	9.24
		Baseline	9.47	9.22

BCVA, best-corrected visual acuity; CS, contrast sensitivity; ETDRS, Early-Treatment Diabetic Retinopathy Study; GCL, ganglion cell layer; HVF, Humphrey visual field; logMAR, logarithm of the minimal angle of deviation; MD, mean deviation; PMB, papillomacular bundle; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD-OCT, spectral-domain optical coherence tomography.

Supplemental Table 4. Logarithm of the Minimal Angle of Resolution (LogMAR) and Humphrey visual field (HVF) Mean Deviation (MD) according to tobacco consumption.

	LogMAR			
Tobacco	**Mean**	**SD**	**Min**	**Max**
Current	1.59	0.41	0.40	2.00
Former	1.43	0.49	0	2.30
Never	1.61	0.48	0.60	2.30
	HVF MD			
Tobacco	**Mean**	**SD**	**Min**	**Max**
Current	-23.26	9.08	-33.96	-2.22
Former	-18.71	10.27	-33.40	-2.06
Never	-23.97	10.39	-34.55	-0.31

SD, standard deviation

Supplemental Table 5. Logarithm of the Minimal Angle of Resolution (LogMAR) and Humphrey visual field (HVF) Mean Deviation (MD) according to alcohol consumption.

	LogMAR			
Alcohol	**Mean**	**SD**	**Min**	**Max**
Current	1.51	0.45	0	2.30
Former	1.54	0.48	0.40	2.30
Never	1.63	0.50	0.60	2.30
	HVF MD			
Alcohol	**Mean**	**SD**	**Min**	**Max**
Current	-21.24	10.03	-34.15	-2.06
Former	-20.87	10.90	-34.55	-0.31
Never	-25.14	9.89	-34.48	-1.65

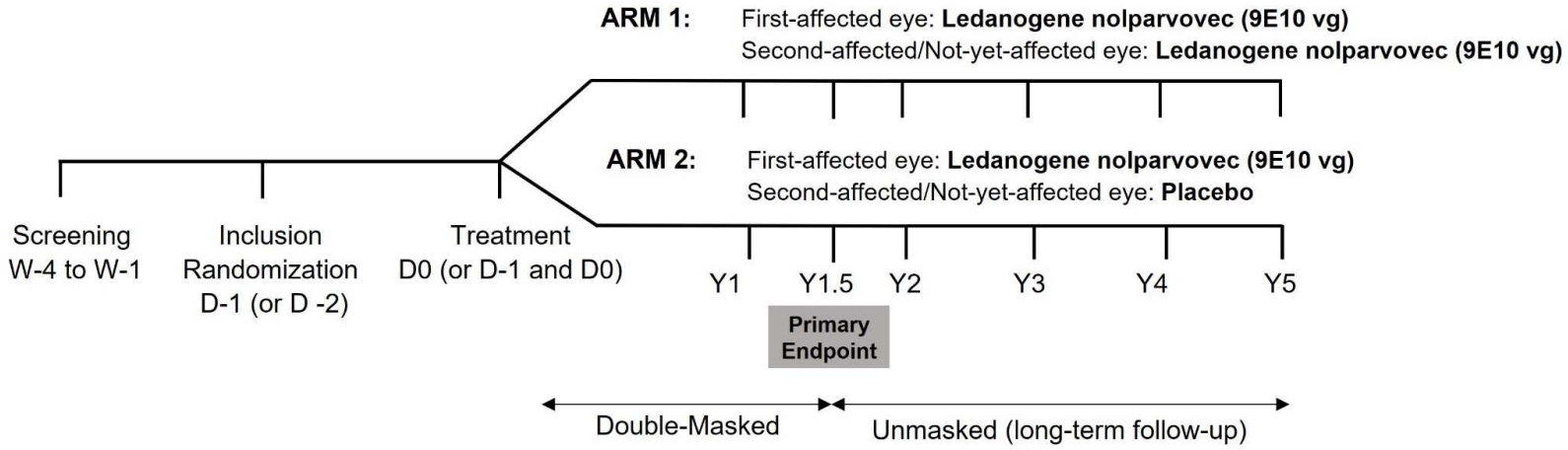
SD, standard deviation

Supplemental Table 6. Individual data of all 5 patients in RESCUE and REFLECT studies with one eye with no vision loss at baseline

			RESCUE				REFLECT
			Subject #12	**Subject #15**	**Subject #22**	**Subject #26**	**Subject**
Age range (years)			20-25	20-25	25-30	30-35	35-40
Gender			F	M	F	M	F
Duration of vision loss in *affected* eye (days)			155	90	57	49	70
Unaffected eye			Left	Right	Left	Right	Right
BCVA (LogMAR) (Snellen)		*Screening*	-0.2 20/13	-0.3 20/10	0.0 20/20	-0.1 20/16	0 20/20
		Inclusion	-0.2 20/13	-0.2 20/13	-0.1 20/16	-0.1 20/16	0 20/20
CS (LogCS)		*Screening*	1.65	1.65	1.65	1.65	1.65
		Inclusion	1.35	1.65	1.5	1.65	1.35
HVF (dB)	**MD**	*Screening*	NR	NR	NR	-1.14	-4.05
		Inclusion	NR	-2.4	-1.9	NR	-2.82
	PSD	*Screening*	NR	NR	NR	1.87	2.44
		Inclusion	NR	1.6	1.29	NR	1.59
SD-OCT	**GCL Macular Volume (mm^3)**	*Screening*	1.06	1.28	1.06	1.27	0.96
		Inclusion	1.07	1.27	1.01	1.24	0.96
	ETDRS Total Macular Volume (mm^3)	*Screening*	8.44	9.53	8.72	9.34	9.24
		Inclusion	8.48	9.63	8.69	9.31	9.22
	PMB RNFL Thickness (µm)	*Screening*	62	118	66	65	63
		Inclusion	64	128	69	69	68
	RNFL Quadrant Temporal (µm)	*Screening*	88	141	81	80	87
		Inclusion	88	154	85	85	90
	RNFL Average Thickness (µm)	*Screening*	107	143	95	118	112
		Inclusion	108	149	97	121	114

BCVA, best-corrected visual acuity; CS, contrast sensitivity; ETDRS, Early-Treatment Diabetic Retinopathy Study; GCL, ganglion cell layer; HVF, Humphrey visual field; logMAR, logarithm of the minimal angle of deviation; MD, mean deviation; NR, not reliable; PMB, papillomacular bundle; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD-OCT, spectral-domain optical coherence tomography.

Supplemental Figure 1. REFLECT study design.



Study treatment was administered on the same day (Day 0) with two separate procedures or on two consecutive days (Day -1 and Day 0) at the investigator's discretion. In all cases, the first intravitreal injection had to be performed the day following inclusion. Subjects were allocated 1:1 to the two arms. If vision loss was simultaneous, one eye was randomly designated as first-affected. D, day; vg, vector genomes; W, week; Y, year